In Vitro Effect of Sorghum (*Sorghum bicolor*) Seed Extracts as a Biological Acaricidal Against Some Hard Tick (Ixodidae) in Sulaimani Governorate - Kurdistan Region/Iraq

Bahzad H.S. Mustafa[1], Kadhm A. Mohammad[2] and Aram O. Mahammed[2]

[1]Department of Animal Production, Faculty of Agricultural Sciences, University of Sulaimani Sulaimani, Kurdistan Region – F.R. Iraq

[2]Department of Agribusiness and Rural Development, Faculty of Agricultural Sciences, University of Sulaimani Sulaimani, Kurdistan Region – F.R. Iraq

Abstract*—This study was conducted in Sulaimani governorate in order to identify the biological control of some Ixodidae genera among different flocks of cattle, sheep and goats. Four genera of Ixodidae; Boophilus spp, Hyalomma spp., Rhipicephalus spp. and Haemaphysalis spp., were identified in these infested animals. According to chi–square test, the highest distribution of Boophulis spp., was recorded in cattle (56.51%), and the highest distribution of Hyalomma spp., (49.82%) and Rhipicephalus spp., (28.16%) which were in sheep. The highest number of Haemophasylas spp., was obtained from goats (6.67%), whereas the lowest number of this genus (2.88% and 2.89%) was collected from cattle and sheep respectively. The toxicity of Sorghum bicolor seed extract was tested against the more distributed Ixodidae genera (Boophilus spp. and Hyalomma spp.) by immersion method on mature ticks, four concentrations (23.2, 17.4, 11.6 and 5.8 mg/dl), in addition to the control treatment (0 mg/dl) of the seed were used to evaluate the engorged females in vitro. The results showed that 100% of absolute cumulative mortality of Boophilus spp., was gain after 72 hr by 23.2 mg/dl extract concentration, followed by 17.4 mg/dl which gave 90% mortality, whereas 100% absolute cumulative mortality for Hyalomma spp., was obtained by 23.2 mg/dl extract concentration after 48 hr, followed by 17.4, 11.6 and 5.8 mg/dl concentration that gave 90%, 80% and 40% mortality after 72 hr.

***Index Terms*—Boophilus spp., Hyalomma spp., Sorghum bicolor seed extract**

ARO-The Scientific Journal of Koya University
Volume IV, No 2(2016), Article ID: ARO.10065, 04 pages
DOI: 10.14500/aro.10065
Received 14 December 2014; Accepted 23 April 2015
Regular research paper: Published 09 September 2016
Corresponding author's e-mail: bahzad.mustafa@univsul.edu.iq

I. Introduction

Ixodidae causes damage directly due to herd irritability, blood spoliation, hide and udder injuries and inoculation of toxins, and indirectly by transmission of *Babesia* spp., and *Anaplasma marginale* especially in cattle and other ruminants (FAO, 2004).

Pesticidal plants have great potential for impact in developing countries (Isman, 2008), but a scientific under-standing of their activity provides opportunities to optimize their use (Stevenson, et al., 2009). Acaricides are needed to control tick infestations and tick-borne diseases. However, the uses of acaricides are constrained by their high costs, tick resistance, concerns about residues in food and in the environment (Mekonnen, 1996). The use of acaricides has disadvantages, such as the selection of resistant tick populations and harmful effects on the animals, human beings and the environment (Garcia-Garcia, et al., 2000) and more toxic and harmful to arthropods than to warm-blooded vertebrates (Okello, et al., 2003). Natural products offer world's resource-poor farmers a cheaper alternative to synthetic acaricides. There is a great potential for the use of Africa's, Asia and South America tropical and subtropical region plants to develop sources of acaricides to reduce the cost of tick control. These plants, being locally available and potentially easy to be produced, locally processed and used by farmers themselves or by cottage industries are an asset for the use of these plants and their extracts by traditional farmers in poor regions of the world. Tick control in the habitat and vegetation requires modification of the plant cover by removal of vegetation that shelters ticks (Baars, 1999). Various stages of some ticks, e.g. *Boophilus* spp. attach themselves to the blades of grass and other vegetation and stealthily attach to the cattle passing nearby. Though clearance of vegetation will annihilate their places of shelter, this type of action, however, may encourage soil erosion and may be detrimental to the ecosystem (Muhammad, et al., 2008). Farmers also used alternative

methods as an herbicidal such as used engine oil (12%), Jeyes fluid (household disinfectant 24%) (Mbati, et al., 2002).

Abdel-Shafy and Zayed (2002) examined the acaricidal effect of plant extract of Neem seed oil (Azadirachta indica) on egg, immature and adult stages of *Hyalomma* spp. and concluded that Neem can be used for tick control at economic concentrations of 1.6% to 3.2%. So, although herbal medicine research in veterinary parasitology is a recent area in most country, it has shown the potential to become a future tool to reduce the problems faced by animal breeders, such as resistance and residues, also prolonging the useful life of commercial chemical products applied for parasite control through the association of bioactive plant substances with synthetic products (Chagas, 2008). Sheep, cattle and goats are very common in Iraq especially in rich areas with green pastures (Hasson, 2012).

II. Materials and Methods

A. Sample of Sorghum (Sorghum bicolor) and Preparation of Extract.

Sorghum Bicolor (seed)

Two hundred grams of *sorghum bicolor* seed (natural product available in Sulaimani market) were cut in to small pieces and crushed using mixer blinder, and then immediately extracted with 300 ml of distilled water for 24 hr and filtrated. The aqueous extracts were serially diluted to $^1/_2$, $^1/_3$ and $^1/_4$ of original solution with distilled water (Sheldrick, 1984), an experimental was carried out in the photochemistry laboratory, Faculty of Agricultural Sciences, University of Sulaimani.

Tick Collection and Identification

Engorged female of ixodidae were collected manually (using cotton and alcohol 70%) from different flocks of cattle, sheep and goat, They were placed individually into clean universal glass vials and kept in room temperature to counted and identified by morphological features according to (Hoogstraal, 1956; Walker, et al., 2003).

B. Laboratory Experiments

Study of the effects of *S. bicolor* extract seed on the activity of hard tick; using four concentration of the extract used for the study, whereas; 23.2 (100%), 17.4 (75%), 11.6 (50%) and 5.8 (25%) was exposed to hard tick (using 10 ml of each concentration of this extract in Petri dish and put the distilled water on one Petri dish as a control (0% concentration) with number of hard tick), The number of engorged female found dead in each plate was recorded and monitored at certain interval of time; 30 min, 1 hr, 2 hr, 4 hr, 6 hr, 12 hr, 24 hr, 48 hr and 72 hr, and the results were summarized.

C. The Statistical Analysis

After collecting the raw data, Statistical tests were performed according to chi-square test as a goodness of fit for the different species of Ixodidae between the studied animals namely; cattle, sheep and goats, and the mean of mortality and cumulative mortality and confidence interval 95% for mortality by the two species (*Boophilus* and *Hyalomma* spp.) were compared, using simple t-test between the two means (steel, et al., 1997).

III. Results

In this study, the overall hard tick 973 collected from 300 hundred isolated in infested animals (one hundred in each; cattle, sheep and goats), as the following, 591 hard tick in cattle, 277 in sheep and 105 in goats.

Table I identifies four genera of Ixodidae; *Boophilus* spp., *Hyalomma* spp., *Rhipicephalus* and *Heamophalus* spp., according to the X^2 (chi- square) test for equally distribution of these species among different studied animals, the results shows highly significant ($P < 0.01$) values of chi-square and they were not equally distribution. The highly distribution of *Boophulis* spp., observed in cattle 334 (56.51%) and in goat 47 (44.76), in sheep the highly distributed showed in *Hyalomma* spp., 138 (49.82%) and *Rhipicephilus* spp., 78 (28.16 %), whilst shows the lowest number of *Haemophasylas* spp. collected in cattle and sheep 17 (2.88), 8 (2.89) respectively.

TABLE I
DISTRIBUTION OF IXODIDAE SPECIES IN CATTLE, SHEEP AND GOAT IN DIFFERENT FLOCKS IN SULAIMANI GOVERNORATE

Animal	*Boophilus* spp. %	*Hyalomma* spp %	*Rhipicephalus* Spp %	*Haemophasylas* spp. %	Total
Cattle	334 (56.51)	174 (29,44)	66 (11.17)	17 (2.88)	591
Sheep	53 (19.13)	138 (49.82)	78 (28.16)	8 (2.89)	277
Goats	47 (44.76)	27 (25.71)	24 (22.86)	7 (6.67)	105

$\chi^2_{Cal.} = 36.61^{**}$

** χ^2_{Cal} *is highly significant at the 0.01 level (2-tailed),* $\chi^2_{0.01}(6) = 16.81$

Data in Table II shows the cumulative mortality % of the *Boophilus* spp., affected by different concentration of *Sorghum bicolor* seed extracts during 72 hr as shown the highest mortality caused by the crude extract which considered as 100% concentrated, while the lowest concentration 5.8 mg/dl or 25% concentration. The crude extract started after 2 hr but the lowest concentrate 25% stared after 48hr only the absolute cumulative mortality 100% shown by the crude extract after 72 hr followed by 90% mortality gained by the 2nd concentration while the 3rd and 4th concentration the mortality declined to 65% and 15% respectively. Increasing the concentration of the extract shows increasing in mortality as an average of the studied time the maximum value was 37.22% gained by the crude extract and the lowest mortality was 2.22% as an average of the fourth concentration. Overall the time the highest confidence intervals ($P = 0.95$) obtained by the crude extract lies between 11.64 - 62.80% followed by the 2nd concentration which was 5.77-56.45%, whilst the 3rd and 4th concentration shows lower levels of confidence intervals under 50%, which consider to be not effective.

Cumulative mortality % of the *Hyalomma* spp., affected by different concentration of *Sorghum bicolor* seed extracts during 48hrs as shown the highest mortality caused by the crude extract which considered as 100% concentrated, while the lowest concentration 5.8 mg/dl or 25% concentration. The crude extract started after 1hr but the lowest concentrate 25% and 50% stared after 2 hr. the absolute cumulative mortality 100% shown by the crude extract 23.2 mg/dl after 48 hr followed by 2nd concentration 90% and the 3rd 80% after 72 hr whilst the 4th concentration the mortality declined to 40% after the same time. Increasing the concentration of the extract shows increasing in mortality as an average of the studied time the maximum value was 48.33% gained by the crude extract and the lowest mortality was 11.66% as an average of the fourth concentration.

TABLE II
EFFECT OF DIFFERENT CONCENTRATION OF *SORGHUM BICOLOR* EXTRACT ON THE MORTALITY PERCENTAGE OF *BOOPHILUS* SPECIES THROUGH 72 HR.

Extract conc. (mg/dl)	Cumulative Mortality/Time (%)									$\overline{X}$	C.I (95%)
	1/2 hr	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	48 hr	72 hr		
Distilled water (Control)	0	0	0	0	0	0	0	0	0	0	0
23.2	0	5	10	20	30	45	55	70	100	37.22	11.64 – 62.80
17.4	0	0	5	10	20	30	55	70	90	31.11	5.77 – 56.45
11.6	0	0	5	5	20	25	45	55	65	24.44	5.33 – 43.56
5.8	0	0	0	0	0	0	0	5	15	2.22	0.00 – 6.11
$\overline{X} = 23.75$											

Overall the time the highest confidence intervals ($P = 0.95$) obtained by the crude extract lies between 18.83 – 77.84 % followed by the 2nd concentration which was 12.55 – 64.12%, whilst the 3rd and 4th concentration shows lower levels of confidence intervals under 50%, which consider to be not effective, this result shown in Table III.

TABLE III
EFFECT OF DIFFERENT CONCENTRATION OF SORGHUM BICOLOR EXTRACT ON THE MORTALITY PERCENTAGE OF *HYALOMMA* SPECIES THROUGH 72 HR.

Extract conc. (mg/dl)	Cumulative Mortality/Time (%)									$\overline{X}$	C.I (95%)
	1/2 hr	1 hr	2 hr	4 hr	6 hr	12 hr	24 hr	48 hr	72 hr		
Distilled water (Control)	0	0	0	0	0	0	0	0	0	0	0
23.2	0	5	15	30	50	60	75	100	---	48.33	18.83 – 77.84
17.4	0	5	10	20	35	45	60	70	100	38.33	12.55 – 64.12
11.6	0	0	5	10	20	30	40	55	80	26.66	5.58 – 47.75
5.8	0	0	5	5	5	10	15	25	40	11.66	1.52 – 21.81
$\overline{X} = 31.25$											

IV. DISCUSSION

Research involving using *sorghum bicolor* seed extract for control hard tick (Ixodidae) *Hyalomma* and *Boophilus* spp., on cattle, sheep and goats flocks in Sulaimani governorate. In vitro tests conducted with *S. bicolar* at 23.2 mg/dl (100%), 17.4 mg/dl (75%), 11.6 mg/dl (50%) and 5.8 mg/dl (25%); showed the highest cumulative mortality after 72 hr in 50% and 100% concentration on *Boophilus* spp., engorged female, In Iraq, this results agreement with Mustafa and Faraj (2013), which observed that the genera *Boophilus* spp., there were highly significant differences between other tick species when collected and calculated by Less Significant Differences test (LSD) in Kurdistan region/Iraq. Several in vitro studies have proven the potential use of medical herbs and their essential oils and isolated substance for control of the tick *Boophilus* spp. Essential oils are complex mixtures containing many tens or even hundreds of substance with varied chemical composition (ISO, 1997). Silva, et al. (2007) obtained extracted product (EP) of 42% on engorged R (*Boophilus*) microplus females of the Alcohol extract of cymbopogon citrates of 100% concentration. Olivo, et al. (2008) observed efficiency of 85% on engorged *Boophilus microplus* females of C. nordus essential oil at 10%. Farias, et al. (2007) obtained 100% efficacy in controlling *R* (*Boophilus*) *microplus* using seed oil of *C. guianesis* at concentration ranging from 10% to 100% and had 100% efficacy against engorged femals of *R. sanguine* and *Amblyomma nitens* (Farias, et al., 2009). Nahar, et al. (2005) revealed that Ata (*Annona retirulata*) and bishkatali (*polygonum hydropiper*) have great acaricidal value against *Boophilus microplus*. Described the *Hyalomma* spp., the widespread species presence between awassi sheep in Iraq and transmitted malignant ovine theileriosis, may be because Awassi sheep move between region to another; losses may be higher in important sheep (brown, et al., 1990). Razmi *et al*. (2003) demonstrated the *Rhipicephalus* spp. and *Hyalomma* spp., were the most common species in sheep and goats in Iran. While Nasiri, et al. (2010) identified two genera and five species of *Hyalomma* and *Haemaphysalis* in mountainous region in Iran. The cumulative mortality of *Hyalomma* spp., as a highest in 100% after 48 hr and 50% after 72 hr, and the sensitivity of *Hyalomma* spp., was more than *Boophilus* spp., may be due to *Hyalomma anatolicum* as a three host tick it may feed as larva or nymphs on gerbils, then on cattle or sheep as adults (Walker, et al., 2003), and attack mutable host for short time for feeding and have not time to expose to the acaricide and this leads to preserved to resistance and remaining for long time to survival, this behavior contrast with *Boophilus* spp., because remains continuously on the host from the moment of access as an unfed larva until its departure as an engorged female. This type of life-cycle is characteristic of *Boophilus* spp. (Minjauw and Mc Leod, 2003), and therefore more exposed to acaricide, according to the t-test, the high mortality % of *Hyalomma* spp., was 31.25% compared to the lower mortality % of *Boophilus* spp., was 23.75%, but their difference was not significant. Godara, et al. (2015) revealed that ethanolic extract obtained from the aerial parts of *Artemisia absinthium* has acaricidal properties and could be useful in controlling, adults, eggs and larvae of *Hyalomma anatolicum*.

V. Conclusions

Sorghum bicolor seed extract more significant (more toxic) effect (100% mortality) after 48 hr with *Hyalomma* spp., while shows the same concentration (23.2 mg/dl) a significant effect on *Boophilus* spp., after 72 hr to killed hard tick (ixodidae). Therefore, using of some plants extract which effected on the ixodidae, especially extract of *sorghum bicolor* seed have many advantages over synthetic chemical, loss toxicity of mammalian and environmental pollution, being less expensive and easily available for control hard tick (Ixodidae).

References

Abdel Shafy, S. and Zayed, A.A., 2002. In vitro acaricidal effect of plant extract of neem seed oil (Azadirachta indica) on egg, immature and adult stages of Hyalomma anatolicum excavatum (Ixodidea: Ixodidae). *Vet. Parasitol*, 106(1), pp.89-96.

Baars, R.M.T., 1999. The effect of rangeland fires on cattle tick infestation in western Zambia. Trop. *Anim. Health. Prod*., 31(5), pp.275-278.

Brown, C.G.D., Hunter, A.G. and Luckins, A.G., 1990. Diseases caused by protozoa, In: Sewell, M.M. H. and Brocklessly, D.W.(eds). Handbook of Animal Diseases in the Tropics. 4th Ed, (University Press Cambridge, U.K.).

Chagas, A.C.S., 2008. Metodologias in vitro para avaliaçao de fitoterapicos sobre parasitas e resultados de testes a campo. In: Congresso Brasileiro de Parasitologia Veterinaria, 2008, Curitiba. Anais do Congresso Brasileiro de Parasitologia Veterinaria. Curitiba: CBPV, 15, p.13.

FAO (Food and Agriculture Organization of the United Nations), 2004. Module 1. Ticks: acaricide resistance: diagnosis management and prevention. In: Guidelines Resistance Management and Integrated Parasite Control in Ruminants. FAO Animal Production and Health Division, Rome.

Farias, M.P.O., Sousa, D.P., Arruda, A.C., Arruda, M,S,P., Wanderley, A.G., Alves, L.C. and Faustino, M.A.G., 2007. In vitro effectiveness of the oil of the Carapa guianensis Aubl. (andiroba) in the control of Boophilus microplus (Acari: Ixodidae). *Rev. Bras. Plantas. Med. Botucat*., 9(4), pp.68–71.

Farias, M.P.O., Sousa, D.P., Arruda, A.C., Wanderley, A.G., Teixeira, W.C., Alves, L.C. and Faustino, M.A.G., 2009. Potencial acaricida do oleo de andiroba Carapa guianensis Aubl. sobre femeas adultas ingurgitadas de Anocentor nitens Neumann, 1987 e Rhipicephalus sanguineus Latreille, 1806. *Arq. Bras. Med. Vet. Zootec*., 61, pp.877–882.

Garcia-Garcia, J.C., Montero, C., Redondo, M., Vargas, M., Canals, M., Bone, O., Rodriquez, M., Joglar, M., Machado, H., Gonzalez, I.L., Valdes, M., Mendez, L. and de la Fuente, J.. 2000. Control of ticks resistant to immunization with Bm86 in cattle vaccinated with the recombinant antigen Bm95 isolated from the cattle tick. *Boophilus microplus. Vaccine*., 18(21), pp.2275-2287.

Godara, R., Parveen, S., Katoch, R., Yadav, A., Katoch, M., Khajuria, J.K., Kaur, D., Ganai, A., Verma, P.K., Khajuria, V. and Singh, N.K., 2015. Acaricidal activity of ethanolic extract of Artemisia absinthium against Hyalomma anatolicum ticks. *Exp. Appl. Acarol*., 65(1), pp.141-148.

Hasson, R.H., 2012. Tick distribution and infestation among sheep and cattle in Baghdad's south suburb. *Kufa J. Vet. Med. Scie*., 3(1), pp.77-90.

Hoogstraal, H., 1956. African Ixodidae. 1. Ticks of the Sudan. U.S. Naval Medical Research Unit No3. Cairo, Egypt.

Isman, M.B., 2008. Perspective Botanical Insecticides: for richer for poorer, *Pest Management Science*, 64, pp.8–11.

ISO - International organization for standardization 1997. Aromatic natural raw materials - Vocabulary: ISO 9235. Genebra, p.8.

Mbati, P.A., Hlatshwayo, M., Mtshali, M.S., Mogaswane, K.R., de Waal, T.D. and Dipeolu, O.O., 2002. Ticks and tick-borne diseases of livestock belonging to resource-poor farmers in the eastern Free State of South Africa. *Exp Appl Acarol*, 28(1-4), pp.217-224.

Mekonnen, S., 1996. Epidemiology of ticks and tick-borne diseases in Ethiopia: Future research needs and priorities. In: ILRI, *The International Livestock Research Institute*. Nairobi, Kenya, 12-13 March 1996, pp.17-29.

Minjauw, B. and Mc Leod, A., 2003. Tick-borne disease and poverty. The impact of ticks and tick-borne disease on livelihood of small –scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme. Edinburg: Center for Tropical Veterinary Medicine, University of Edinburgh, UK, pp.1-116.

Muhammed, G., Naureen, A., Firyal, S. and Saqib, M., 2008. Tick control strategies in dairy production medicine. *Pak. Vet. J.*, 28(1), pp.43-50.

Mustafa1, B.H.S. and Faraj, S.H., 2013. Resistance of Hard Tick (Ixodidae) with some Acaricide in Cattle (Naturally Infestation) in Sulaimani Governorate Fields-Kurdistan Regional/Iraq. *J. Agri. Sci. Techno*., A3, pp.927-934.

Nahar, L., Anisuzzaman., Alim, M.A., Karim, M.J., Islam, K.I. and Mondal, M.M.H., 2005. In vitro acaricidal effects of some indigenous plants against Boophilus microplus (Aracnida: Ixodidae). *Bangladesh.J.Vet.Med*., 3(2), pp.118-123.

Nasiri, A., Telmadarraiy, Z., Vatandoost, H., Chinikar, S., Moradi, M., Oshaghi, M.A., Salim abadi, Y. and Sheikh, Z., 2010. Tick Infestation Rate of Sheep and Their Distribut ion in Abdanan County, Ilam Province, Iran, 2007-2008. *Iranian. J. Arthropod-Borne. Dis*., 4(2), pp.56-60.

Okello. O.J., Tukahiriwa, E.M., Perry, D.B., Rowlands, G.J., Nagda, S.N., Musisi, G., Bode, E., Heinonen, R., Mwayi, W. and Opuda, A.J., 2003. The impact of tick control on the productivity of indigenous cattle under ranch condition in Uganda. *Trop. Anim. Health. Prod.*, 35(3), pp.237-247.

Olivo, C.J.l; Carvalho, N.M.; Da Silva, J.H.S.; Vogel, F.F.; Massariol, P.; Meinerz ,G.; Agnolin, C.; Morel, A.F. and Viau, L.V. (2008). Oleo de citronela no controle do carrapato de bovinos, *Cienc Rural*. 38, pp.406–410.

Razmi, G.R.; Naghibi, A.; Aslani, M.R.; Dast jerdi, K. and Hossieni, N. 2003. An epidemiological study On Babesia infection in small ruminants in Mashhad suburb, Khorasan province, Iran. *Small Ruminant Res*., 50(1), pp.39-44.

Silva, W.W., Athayde, A.C.R., Rodrigues, O.G., Araujo, G.M.B., Santos, V.D., Neto, A.B.S., Coelho, M.C.O.C. and Marinho, M.L., 2007. Effects of neem (Azadirachta indica A.Juss) and lemongrass [Cymbopogoncitratus(DC)Stapf]Boophilusmicroplus and Rhipicephalus sanguineus (Acari: Ixodidae) in the semiarid region of Paraiba State, Brazil. *Rev. Bras. Plant. Med. Botucatu*, 9(3), pp.1–5.

Sheldrick, B.H., 1984. Analytical methods manual. Land Resource research in states Ottawa, Ontario, LRRI contribution, pp.30-84.

Steel, R.G.D., Torrie, J.H., and Dickey, D.A., 1997. *Principles and procedures of statistics: A biometrical approach*. 3rd ed. Hill book Co. Inc. New York, pp.400-428.

Stevenson, P. C., Dyarathna, T. K., Belmain, S. R. and Veitch, N. C., 2009. Bisdemosidic saponins Securidace longepeduncu Lata Coleaptere (Polygalaceae) with deterrent and toxic properties to storge pests, *J. Agricul. F. Chemistry*., 57, pp.8860-886.

Walker, A.R., Bouattour, A., Camicas, J.L., Estrada-Pena, A., Horak, I.G., Latif, A., Pegram, R.G. and Preston, P.M., 2003. Ticks of domestic animals in Africa, A guide to identification of species. Bioscience. Reports. U.K, pp.86-214.